IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:
Daryn KENNY et al.

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Examiner: Jeanine Anne GOLDBERG

Title: HIGHLY SENSITIVE GENE DETECTION AND LOCALIZATION USING IN SITU BRANCHED-DNA HYBRIDIZATION

DECLARATION UNDER OF INVENTOR DARYN KENNY, PH.D.

IN SUPPORT OF THE APPLICATION

I, Daryn Kenny, declare:

1. I am an inventor of the patent application identified above.

2. I have a Ph.D. in Biological Sciences from the University of California, which I received in 1995.

3. I am a Senior Staff Scientist at Bayer Diagnostics, located at 725 Potter Street in Berkeley, California. I have been with Bayer since 1999. I have been a Senior Staff Scientist since 2002. Previously, at Bayer I worked as a Staff Scientist from 2000 to 2002, and as a Senior Research Scientist from 1999 to 2000.

4. I have read the final Office Action dated September 10, 2004, and understand its contents. I have also read and am familiar with the references cited in the Office Action, which are as follows:

- Antao et al., *In Situ Hybridization Using the bDNA Technology*, TECHNIQUES IN QUANTIFICATION AND LOCALIZATION OF GENE EXPRESSION, Ch. 6, pp. 81-93 (1999);
- Xu et al., *In Situ Hybridization of mRNA with Hapten Labeled Probes*, IN SITU HYBRIDIZATION, Ch. 4, pp. 87-106 (1999);

- Schaeren-Wiemers et al., *A Single Protocol to Detect Transcripts of Various Types and Expression Levels in Neural Tissue and Cultured Cells: In Situ Hybridization Using Digoxigenin-Labeled cRNA Probes*, HISTOCHEMISTRY 100:431-440 (1993);
- Cao et al., *A Sensitive, Rapid, and Non-Isotopic In Situ bDNA Assay for Detection of hnRNPA2 mRNA*, ABSTRACT #2287, March 1998;
- Nolte, *Branched DNA Signal Amplification for Direct Quantitation of Nucleic Acid Sequences in Clinical Specimens*, ADVANCES IN CLINICAL CHEMISTRY 33:201-235 (1998);
- Decimo et al., *In Situ Hybridization of Nucleic Acid Probes to Cellular RNA*, GENE PROBES 2: A PRACTICAL APPROACH, pp. 183-198 (1996);
- Sarto et al. (U.S. Patent No. 6,022,689);
- Kern et al., *An Enhanced Sensitivity Branched-DNA Assay for Quantification of Human Immunodeficiency Virus Type 1 RNA in Plasma*, JOURNAL OF CLINICAL MICROBIOLOGY 34(12):3196-3202 (1996); and
- Siadat-Pajouh et al., *Introduction of a Fast and Sensitive Fluorescent In Situ Hybridization Method for Single-Copy Detection of Human Papillomavirus (HPV) Genome*, JOURNAL OF HISTOLOGY AND CYTOCHEMISTRY 42(11):1503-1512 (1994).

5. The Examiner is of the position that the claimed invention is obvious over various combinations of the cited references. The following discussions, which focus on the Examiner's rejections of this application's independent claims, will explain why the Examiner's obviousness analysis is not correct.

6. The present invention is unique over the art available at the time of the invention because the present invention allows for the detection of very small quantities of DNA *in situ* using bDNA hybridization for signal amplification. Prior to the invention, the bDNA assay was only capable of detecting RNA *in situ*. Because expression levels of RNA are significantly greater than that of DNA, *in situ* RNA assays do not require the sensitivity that *in situ* DNA assays require. An inherent problem encountered in all *in situ* DNA assays is background noise. Because RNA has significantly higher expression levels than DNA, the signal of the RNA is capable of being seen over the background noise. The reason for the high background noise is that large quantities of oligonucleotides are required in order to perform the bDNA assay. The present invention is aimed specifically at overcoming these obstacles inherent in the application of *in situ* bDNA assays to test for DNA in whole tissue samples, to detect the subcellular localization of nucleic acid analytes within a sample of biological material, and to detect 1-10

copies of a nucleic acid analyte in biological material. A detailed discussion of the problems previously encountered in bDNA *in situ* hybridization assays and the advantages that the present invention has had in overcoming the previous problems are discussed in Kenny et al., *Detection of Viral Infection and Gene Expression in Clinical Tissue Specimens Using Branched DNA (bDNA) In Situ Hybridization*, J. HISTOCHEM. CYTOCHEM. 50(9):1219-1227, 1220, 1225-1126 (2002) (attached).

7. The Examiner rejects claims 1, 3-4, 6-23, and 27-33 over Antao et al. in view of Xu et al. (Office Action, pages 3-7). It is the Examiner's position that the hypothetical combination of the bDNA *in situ* RNA assay described in Antao et al. and the washing step described in Xu et al. results in the claimed invention. The combined teachings from Antao et al. and Xu et al. would not cause one of ordinary skill in the art to arrive at the claimed invention for the following reason.

8. At the time of the invention, myself and the other inventors of this application applied the reaction conditions for RNA *in situ* hybridization using bDNA for signal amplification as described in Antao et al. and Xu et al. to screen for DNA and the results were not satisfactory. The background noise in th reactions was such that DNA signals could not be seen. Because of the inability of the RNA assays described in Antao et al. and Xu et al. to provide adequate use of a bDNA *in situ* assay for the detection of DNA, myself and the other inventors undertook to correct the deficiencies in the prior art – the result being the invention described in the instant application.

9. The Examiner also rejects claims 1, 3-4, 6-13, 16, 17, and 20-27 over Schaeren-Wiemers et al. in view of Cao et al., Nolte, Decimo et al., and Xu et al. The combined teachings of Schaeren-Wiemers et al. in view of Cao et al., Nolte, Decimo et al., and Xu et al. would not cause one of ordinary skill in the art to arrive at the claimed invention for the following reasons.

10. Like Antao et al. and Xu et al., all of the combined teachings from the Examiner's cited references would only lead the ordinary artisan to experiments relating to the detection of *in situ* RNA. Of all the references, only Nolte suggests that that the bDNA assay may be used for DNA *in situ* hybridization assays; however, because Nolte does not *describe* any such assays, one of ordinary skill in the art would be left to try to use the RNA *in situ* bDNA assays to detect DNA and as mentioned above in paragraph 8, would be left with experimental results rife with background noise and no signal.

11. With respect to the Examiner's suggestion at pages 10-11 of the Office Action that the concentrations of the cRNA probes of Schaeren-Wiemers et al. and Xu et al. may be substituted for the bDNA oligonucleotides, I submit that the ordinary molecular biologist would never reasonably undertake such a substitution. The cRNA probes of Scharen-Wiemers et al. are DIG-labeled probes; the purpose of the claimed invention was specifically to enhance the sensitivity of *in situ* assays by substituting the traditional radiolabeled and DIG-labeled probes previously used for the more highly sensitivity bDNA assay, which uses a number of different target probes. To achieve sufficient sensitivity, at least 12 different probes must be used in the bDNA assay, a number far in excess of the single DIG-labeled cRNA probe disclosed is Schaeren-Wiemers et al.

12. With respect to the Examiner's position that the subject matter of claim 27 is evident from the mere detection of the nucleic acid within the cell at page 11 of the Office Action, I note that the Examiner's position is ***not*** accurate. The detection of the precise location of a nucleic acid analyte within a cell, be it RNA or DNA or a fragment thereof, is not readily apparent from the mere detection of a signal. Because autoradiography and chemiluminescence produce signals that radiate outwards, identifying the position of a nucleic acid analyte within a cell is not possible using these traditional means of signal detection. Through the use of bDNA under the precise conditions set forth in the instant application, myself and the other inventors on this application were able to refine signal amplification such that the background noise created from traditional radiolabelling and chemiluminescence was not longer a hindrance to the identification of the precise location of a nucleic acid analyte within a cell.

13. At page 16 of the Office Action, the rejects claims 28-35 over Siadat-Pajouh et al. in view of Cao et al., Nolte, Decimo, and Xu et al. The combined teachings of Siadat-Pajouh et al. in view of Cao et al., Nolte, Decimo, and Xu et al. would not cause one of ordinary skill in the art to arrive at the claimed invention for the following reasons.

14. Siadat-Pajouh et al. teaches the identification of one to five copies of HPV-DNA using DIG-tail-labeled oligonucleotide probes. The results of Siadat-Pajouh et al. show that of the five probes tested in the fluorescence-based *in situ* hybridization ("FISH") assays, only the DIG-tail-labeled oligonucleotide probes were capable of producing a reproducible result. This reference accurately reflects the difficulty that is inherent in the detection of *in situ* DNA.

15. The Examiner is of the opinion that one of ordinary skill in the art would readily be able to substitute the bDNA techniques disclosed in Cao et al., Nolte, Decimo, and Xu et al. for the FISH assay of Siadat-Pajouh et al. with a reasonable expectation of success. As previously noted, the bDNA assay requires large amounts of oligonucleotide probes to be successful. Whenever large amounts of probes are used, increased background signal is an expected by-product. Because each of the techniques in the secondary references relate to the screening of high expression RNA, background signal is not considered as problematic as it is with low expression DNA; thus, it follows, that the ordinary artisan would not have a reasonable expectation of success in detecting *in situ* DNA merely by substituting the procedures of Cao et al., Nolte, Decimo, and Xu et al. for the DIG-tail-labeled oligonucleotide used in the FISH assay of Siadat-Pajouh et al.

16. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001 and that such willful false statements may jeopardize the validity of the instant application or any patent issuing thereon.

Dated: 8 Dec 04

Daryn Kenny

Daryn Kenny, Ph.D.